

Figure 1: Northern blot analysis of the expression of the cysteine proteinase (CcCP1) gene in different tissues of *Coffea arabica*.

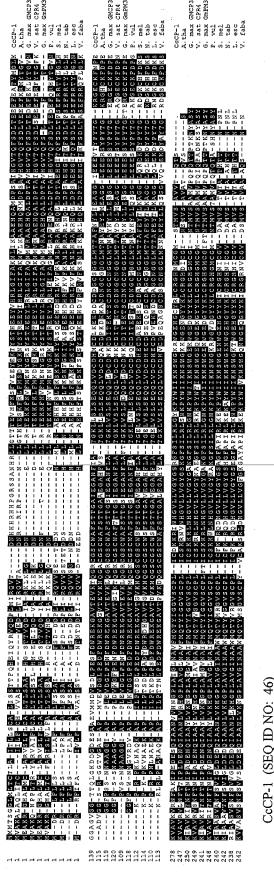
Inventor: McCarthy, et al. App. No.: 10/559,986 Docket No.: 112701-667 REPLACEMENT DRAWING

 $R \hspace{1cm} S \hspace{1cm} L \hspace{1cm} F \hspace{1cm} SG(g) \hspace{1cm} SG(p) \hspace{1cm} LG(g) \hspace{1cm} LG(p) \hspace{1cm} Y(g) \hspace{1cm} Y(p) \hspace{1cm} Red(g) \hspace{1cm} Red(p)$



Figure 2A: Northern blot analysis of the expression of the Cysteine proteinase CcCP-1 gene in different tissues of *Coffea arabica*.

Inventor: McCarthy, et al. App. No.: 10/559,986 Docket No.: 112701-667 REPLACEMENT DRAWING



A.tha (SEQ ID NO: 47)
G. max GMCP3 (SEQ ID NO: 48)
V. sat CPR4 (SEQ ID NO: 49)
G. max GmPM33 (SEQ ID NO: 50)
P. vul (SEQ ID NO: 51)
S. mel (SEQ ID NO: 52)
N. tab (SEQ ID NO: 53)
L. esc (SEQ ID NO: 54)
V. faba (SEQ ID NO: 55)

Figure 2B: Alignment of the full sequence of the protein encoded by CcCP-1 cDNA with other full-length cysteine proteinases available in the NCBI database.

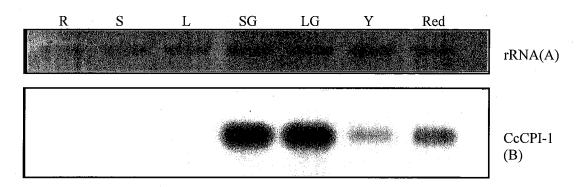


Figure 3: Northern blot analysis of the expression of the cysteine proteinase inhibitor (CcCPI-1) gene in different tissues of *Coffea arabica*.

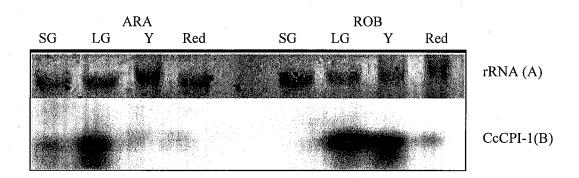


Figure 4: Northern blot analysis of the expression of the cysteine proteinase inhibitor gene (CcCPI-1) at different cherry development stages for *Coffea arabica* (ARA) and *Coffea canephora* (ROB).

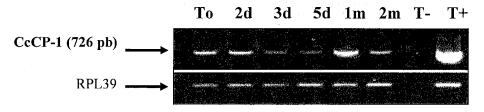


Figure 5. RT-PCR analysis of the expression of *CcCP-1* during *Coffea arabica* grain germination.

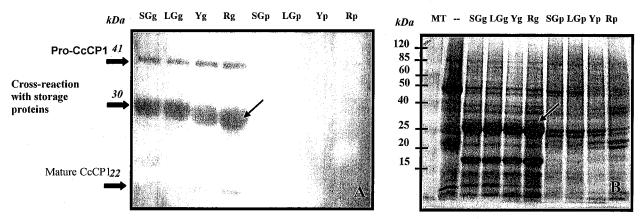
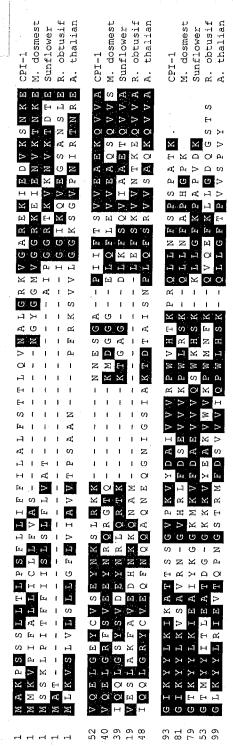


Figure 6A: Western-blot analysis of the expression of CcCP1 protein (A).



CPI-1 (SEQ ID NO: 56)
M. dosmest (SEQ ID NO: 57)
Sunflower (SEQ ID NO: 58)
R. obtusif (SEQ ID NO: 59)
A. thaliana (SEQ ID NO: 60)

Figure 6B: Optimal alignment of the complete protein encoded by CcCPI-1 cDNA with other homologous full-length cysteine proteinases available in the NCBI.

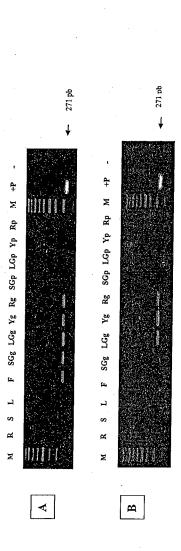


Figure 7: RT-PCR analysis of the expression of CcCPL-1 gene in different tissues of Coffea arabica CCCA2 (A) and Coffea robusta FRT-32 (B).

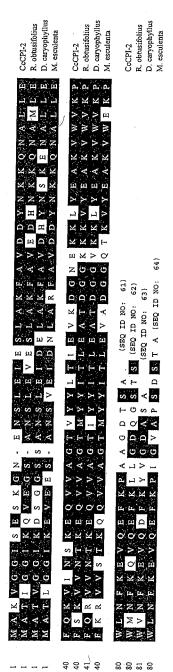


Figure 8: Optimal alignment of the complete protein encoded by CcCPI-2 cDNA with other homologous full-length cysteine proteinases available in the NCBI.

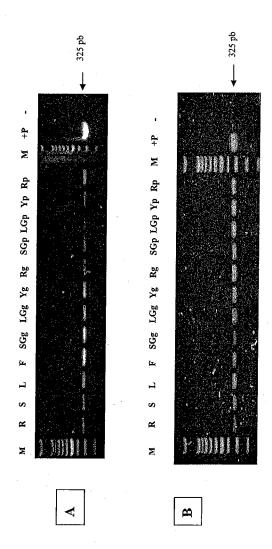


Figure 9: RT-PCR analysis of the expression of CcCPI-2 gene in different tissues of Coffea arabica CCCA2 (A) and Coffea robusta FRT-32 (B).

| CcCPI-3 Citrus x paradisi A. deliciosa A. thaliana | CcCPI-3 Citrus x paradisi A. deliciosa A. thaliana | CcCPI-3 Citrus x paradisi A. deliciosa A. thaliana | CcCPI-3 Cirus x paradisi A. deliciosa A. thaliana |
|---|---|---|--|
| H LELLE T T L A A I C L F S D V P S A A L G G R P K D A L V G G W C L I V L F L S V V P L L A A G D R . K G A L V G G W C S L L L F L L L A L S A A V V G G R . K L V A A G G W A F L L L L L L L L L L L L L L L L L L | - K A D P K P P P B V L B N G K F A I D B H N K B A G T K L B F K T V V E A Q K P I E D P K E K H V M E I G Q F A V T B Y N K Q S K S A L K F E S V E K G B T P I E S L N S A B V Q D V A Q F A V S B H N K Q A N D E L Q Y Q S V V R G Y T P I S N V T D P Q V V B I G E F A V S B Y N K R S E S G L K F E T V V S G B T | Q V V A G T N Y K I V I K A L D G T - A S N L Y E A I V W V K P W L K F K K L T Q V V S G T N Y R L I L V V K D G P - S T K K F E A V V W E K P W E H F K S L T Q V V A G T N Y R L V I A A K D G A - V V G N Y E A V V W D K P W M H F R N L T O V V S G T N Y R L K V A A N D G D G V S K N Y L A I V W D K P W M K F R N L T | |
| O | A A A A A A A | b В У Б Н | |
| | 41 S - K A D P K K D P B V L E N G K F 33 K P 1 E D P K E K H V M E L G Q F S 33 R P 1 E S L N S A E V Q D V A Q F S S P 1 S N V 1 D P Q V V E L G E F | 83 Q V V A G T N Y K 1 V 1 K A L D G 73 Q V V S G T N Y R L 1 L V V K D G 73 Q V V A G T N Y R L V 1 A A K D G 72 Q V V S G T N Y R L V 1 A A K D G | 19 |

Figure 10: Optimal alignment of the complete protein encoded by CcCPI-3 cDNA with other homologous full-length cysteine proteinases available in the NCBI.

| CcCPI-4 Citrus x paradisi A. thaliana | CcCP1-4 Citrus x paradisi A. thaliana | CcCPI-4 Cirrus x paradisi A. tbaliana |
|---|---|---|
| $\begin{array}{cccccccccccccccccccccccccccccccccccc$ | M L A I K T Q D L T R L I L V V K D G P R L K V A A N D G D | VO: 69) NO: 70) (SEQ ID NO: 71) |
| G L S S T W P P R S S T V R K G A L W G G W K P 1 F I E | L N V B Y G F W W I D D D T Y Y B S Y B K G B T Q Y V S G T N Y B T V Y Y S G T N Y B T V Y Y S G T N Y | Y N H N N K (SEQ ID D R K P. M V K (SEQ ID D F E P A N N G R F L |
| G A G Q K N M V G G G G G G S V N V G G G G G G G G G G G G G G G G G | A G T T V V W K S A L K F E S G L K F | K P W E H - F K S L T S K K K F P W M K - F R N L T S S L T S S C T S C T S |
| M N Q R F C C L I V L F L S V V P L L A A G D D M N T S K V V F L L L S L - V V L L L T P L Y A A G D I Y A A G D | Y N A X X Y N X X Y X X X X X X X X X X X X X | G T H C D V A L V R E T C S T K K F E A V V W E T C C V S K N Y L A I V W D D C |

Figure 11: Optimal alignment of the complete protein encoded by CcCPI-4 cDNA with other homologous full-length cysteine proteinases available in the NCBI.

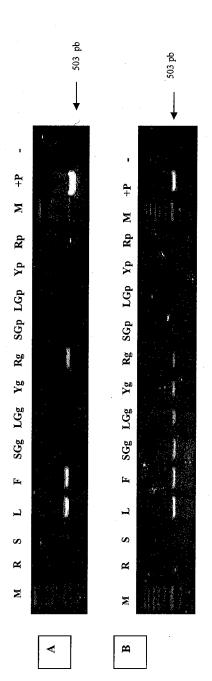
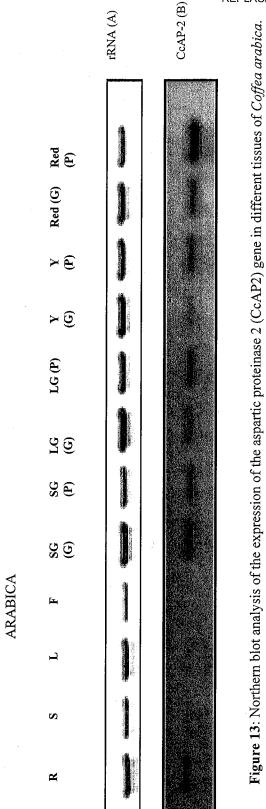


Figure 12: RT-PCR analysis of the expression of CcCPI-4 gene in different tissues of Coffea arabica CCCA2 (Panel A) and of Coffea robusta FRT-32 (Panel B)



Inventor: McCarthy, et al. App. No.: 10/559,986 Docket No.: 112701-667 REPLACEMENT DRAWING

1 | Gettacaletetasatectegate And SAN AND GGG AND GGG AND GGG AND CTT TOT GT GT GT GT GT ATT GGT GGG AND GGG AN

Figure 14: cDNA sequence and its deduced amino acid sequence of CcCP-4. Lowercase: 5' and 3' non-translated regions; Uppercase: Open reading frame; Bold character: amino acid sequence; *: stop codon.

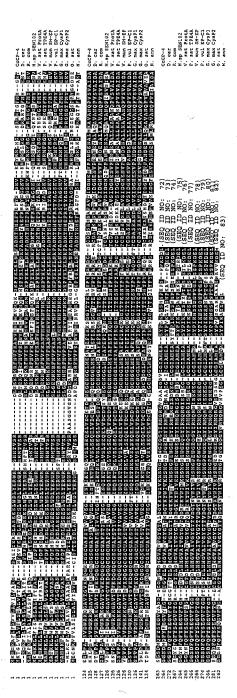


Figure 15: Alignment of the full sequence of the protein encoded by CcCP-4 cDNA with other full-length cysteine proteinases available in the NCBI database

| REFLACEMENT DISAWING | | | | | | | | | | | | | | |
|---|--|---|---|---|-----------------|--|---|---|--|---|--|---|---|---|
|)))) | 000 | 000 000 000 000 000 000 000 000 000 00 | ္ ပု ပု | ပ္ပ ပို ပို |))) |) () () () | 000 | 0 0 0 0 0 |)))) |)))) |))) | υ υ Ο Ο | 9 9 | |
| X DDL | KDEL | KDEL | KDEL | KDEL | KDEL | KDEL | KDEL | KDEL | KDEL | KDEL | KDEL | KDEL | KDEL | |
| GGCTTACATCTTAAATCCTGATTTTATAGATTCGCCTTTCGTGAAGTTCAATCTTCGCAGTCGCTCACTAACATTTGGTAGACATACTTCGATTATGAA | NI AATGGGGAAGGCTTTCCTTTTGCCGTTGTATTGGCTGTGATCTTAGTGGCGGCTATGAGGAGGAAGAAGATTGGCTTTGGCTTCTGAGGAAGC | TEGIGGGACTIGTACGAAGATGGAGGAGCCATCATACTGTTTCTCGAGACCTTTCTGAGAAACGAAAGCGCTTTAATGTTTTCAAGGCAAATGTCCATC | ACATTCACAAGGTGAACCAGAAGGAAGAGCCTTACAAGCTGAAACTCAACATTCGCTGATATGACCAACCA | O GGTGAACATTACCGGATGCTCCACGGCAGTCGTGGTATACTGGATTTATGCATGGGAAGACTTGCCAGCCTCCGTTGATTGGAGAAGAAGTTTGCAAGAAAGA | | AATTAGTTTCTCTGTGCGAGCAAGTACTTGTTGACTGTGAAACGGACAATGAAGGATGCAACGGAGGACTCATGGAAAATGCATACGAGTTTATTAAGAA)] aattagtttctctgtccgagcaagtagttgttgactgtgaaacgacaatgaaggaag | 4 AAGTGGGGGAATAACAACTGAGAGGCTATATCCCTACAAGGCAAGATGGCAGCTGTGATTCATCAAGATGAATGA | 4 CATGAAATGGTACCCGCAAACGATGAGAATGCCTTGATGAAGCTGTTGCTAACCAGCCTGTATCAGTAGCTATAGATGCGTCTGGCTCTGACATGCAAAT Di catgaaatggtacccgcaaacgatgagaatgccttgatgaaagctgttgctaaccagcctgtatcagtagctatagatgcgtctggctctgacatgcaat | 4 ITTATTCAGAGGGTGTATACACTGAGACTCATGTGGCAATGAGCTTGATCATGGCGTGGCGGTCGTCGCTATGGAARCTGCTCTTGACGGTACTAAATA Di tttattcagagggtgtatacgctggagactcgtgtgggcaatgagcttgatcatgggggggg | 474 CTGGATAGTGAAGAACTCATGGGGAACAGGATGGGGAGAACAGGGCTATATCAGGATGCAACGTGGTGTTGATGCTGCTGAAGGCGGAGTTTGTGGGGAATA 1001 CTGGATAGTGAAGAACTCATGGGGAACAGGATGGGGGAAACAGGGCTATATCAGGATGCAACGTGGTGTTGATGCTGATGCTGAAGGCGGAGTTTGTGGGAATA | 574 GCAATGGAGGCCTCCTATCCACTTAAATTGTCCTCCCACAAATCCAAACCATCCCCACCTAAGGACGAGGTCTAGATTGATCCTTATATATA | 674 CATATATATATATATATATATATTTTCRITCATTGAATTTTTAGTTACAGACTACGCGCTTNTGAAGACTTAGATCATCTCTTAGGCATAGATTATAGATTATAG 1201TATATATATATATATATATGATTCATTGAATTTTAGTTAG | 774 TAATCCTGCTCCTGTGATGGTTTGAATAAATAAGTAGTACCNTNTBAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA | Decoration 'Decoration #1': Shade (with solid black) residues that match KDDL -CcCP4 exactly. |
| - | 1 101 | 1 201 | 1 301 | 1401 | 1 501 | 74 | 174 701 | 274 801 | 374 901 | 47 | 5.7 | 123 | 7.7 | ď |

Figure 16. The full length cDNA sequence CcCP-4 KDDL and the partial cDNA sequence CcCP-4 (KDEL)

| 1 81 1 161 17 241 97 | MKMGKAFLFAVVLAVILVAAMSMEITERDLASEESLWDLYERWRSHHTVSRDLSEKRKRFNVFKANVHHIHKVNOKDKPY KLKLNSFADMTNHEFREFYSSKVKHYRMLHGSRANTGFMHGKTESLPASVDWRKQGAVTGVKNQGKCGSCWAFSTVVGVE GINKIKTGQLVSLSEQELVDCETDNEGCNGGLMENAYEFIKKSGGITTERLYPYKARDGSCDSSKMNAPAVTIDGHEMVP GINKIKTGQLVSLSEQELVDCETDNEGCNGGLMENAYEFIKKSGGITTERLYPYKARDGSCDSSKMNAPAVTIDGHEMVP ANDENALMKAVANQPVSVAIDASGSDMQFYSEGVYAGDSCGNELDHGVAVVGYGTALDGTKYWIVKNSWGTGWGEQGYIR ANDENALMKAVANQPVSVAIDASGSDMQFYSEGVYAGDSCGNELDHGVAVVGYGTALDGTKYWIVKNSWGTGWGEQGYIR MQRGVDAAEGGVCGIAMEASYPLKLSSHNPKPSPPKDDL (SEQ ID NO: 86) | CCCP-4 KDDI CCCP-4 KDEI |
|--|---|---|
| Decoi | Decoration 'Decoration #1': Shade (with solid black) residues that match CcCP-4 KDDL exactly. | |

Figure 17. The complete open reading frame of CcCP-4 (KDDL) and the partial open reading frame of CcCP-4 (KDEL).

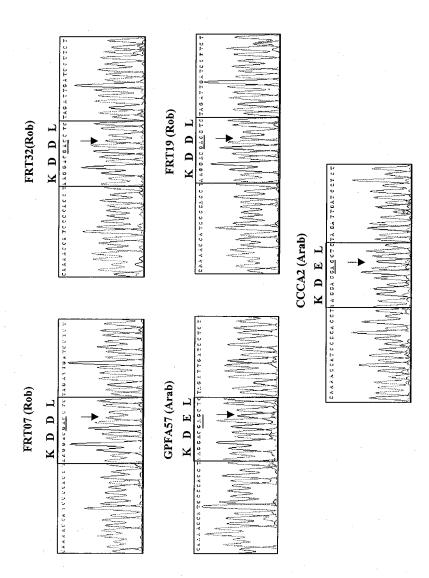


Figure 18. DNA sequence chromatograms for PCR amplified genomic DNA encoding the KDEL/KDDL region of the CcCP-4 gene.

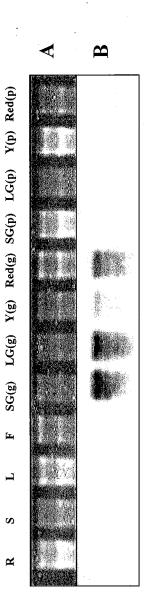


Figure 19. Northern blot analysis of the expression of the Cysteine proteinase CcCP-4 gene in different tissues of Coffea arabica.

App. No.: 10/559,986
Docket No.: 112701-667
REPLACEMENT DRAWING

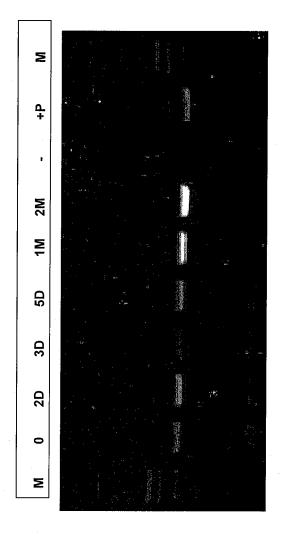


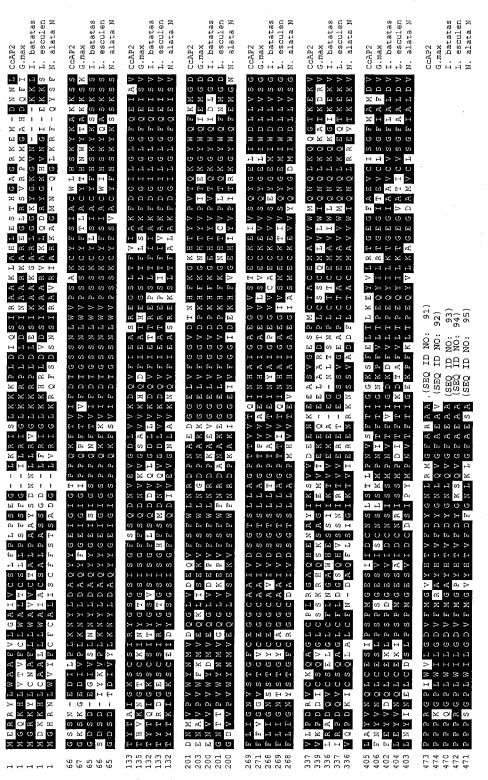
Figure 20. RT-PCR analysis of the expression of CcCP-4 in the whole grain during germination.

| (AY09961 (BAB0930 | Y09961 AB0930 | (AY09961 (BAB0930 | (AY09961 (BAB0930 | (AY09961 (BAB0930 | Y09961 |
|--|---|--|--|---|---|
| SAPI PATha (A PATha (B | CcAP1 AP A Tha (A AP A Tha (B | CcAP1 AP A Tha (A AP A Tha (B | CcAP1 AP A Tha (A AP A Tha (B | CcAP1 AP A Tha (A AP A Tha (B | CcAP1 AP A Tha (AY09961 |
| SONFVFNVTHKFAGKEKQLSELKSHDSFRHARMLANIDLPLGGDSRADSIGLVFTKINGAPATA | A A A H M A | | | | |
| | S H | X X X | 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 | R E S I | |
| <u>.</u> | P C | K V K | P D D | 8 X Q 0 0 | HZ. |
| 4 , D | 5 . 4 2 . 4 | 8 0 0 8 0 0 9 0 0 | \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ | M 1 0 0 1 | N T T |
| - , O | 0 . D | 7700 | T T T E | N N N | |
| ο, ο Δ, Δ | W . A | 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 | 1 D S | G W F G W |] A - |
| 5 . D | Z 1 F | N N N N N N | 6 4 1 5 6 4 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 | 0 0 0 | A L R |
| 7 . I | C L S | F 6 0 | S G S G G S | H D D R E D R E D | 2 H G |
| < , Z < , < - , , , , , , , , , , , , , , , , , , , | 0 0 c | 1 | 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 | 7 7 7 7 0 0 7 0 | Z Z Z |
| , , ¤ | T C D | V D G V D G | N N N N N N N N N N N N N N N N N N N | 1 A 2 1 A 3 | S N S S |
| , , , , | 7 . X | T C A A D S A A | 8 8 1 1 0 8 8 1 1 0 1 1 1 1 1 1 1 1 1 1 | / Y P R | . Z |
| , , O H K | S - S - S | ο ο ο ο ο ο ο | V L E | N A A | S S H H |
| , Lu | M K A | S G B | υ υ υ υ υ ο ο ο ο | D S L S V S V S V S V S V S V S V S V S V | 4 t C |
| 0 L S | L Y D | 0 0 0 0 x z | A I E A I E G M D | H F E D H F E | 2 N N N N N N N N N N N N N N N N N N N |
| x | D , A | S 0 0 0 | Z Z Y | <u>t.</u> _] × ⊢ Z > > > | 国 E C |
| ĕ | 1. 6-1 | 1 V · F V V · F | > > > > | > L > | L R D |
| H / | S S N N N N N N N N N N N N N N N N N N | A A G | 2 | 0 0 0 0 X A | S 1 0 0 |
| z | 2 X | 8 S A | I V P I | N K N | N N N |
| , , <u>,</u> , , z | A G C V R C P K W S S L G I D L T L Y D M K A S S T G R L W T C D Q D P C L S A P N A P A S D C R V G N P C A Y S W T Y A P C P K C P V K T D L G I P L S L Y D S K T S S T S K N W G C E D D P C S - F I M Q S E T C G A K K P C S Y H W V Y | G N L Q T I P M N G S I V F G C S S Q Q S G E L G S S T E A V D G I L G F G Q A N S S I I. S Q L A S A G K V K K I F S H G N R Q T G S T N G T I I F G C G S K Q S G Q L G E S Q A A V D G I M G F G Q S N S S F I S Q L A S Q G K V K R S F A H G N L R T A P L A Q E V V F G C G K N Q S G Q L G Q T D S A V D G I M G F G Q S N T S I I S Q L A A G G S T K R I F S H | KTTPLVPNEAHYNVVLNATENGODVLNLPSDVLGGSGSGTTIDSGTTAAYLPDDVYTPL KTTPNLSKSAHYSVNLNATEVGNSVLELSSNAFDSGDDKGVI DSGTTLVYLPDAAVNPL KTTPI VPNQVHYNVI LKGMDVDGDPI DLPPSLASTNGDGGTT DSGTTLAALPQNLYNSL | CFVYSGNVDDGFPVVXFHFEDSLSLTVYPHEYDFDHDDQWCLGWQNKGMQTRDGREVTL CFHYTDKLDR-FPTVTFQFDKSVSLAVYPREYLFQVREDTWCFGWQNGGLGTKGGASLTI CFSFTSNTDKAFPVVNLHFEDSLKLSVYPHDYFFSLREDMYCFGWQSGGWTTQDGADVIL | WAETNOSSSIKLRDEKSGNVYAVOSH - IISSARGLNAGKALRFLLIITSLLHALLIP WINHNOSGOLOVRDESSAN YVOCH-NISWSSIAITKLIIVSLIPPENVAL WADHNOSSSIKVEDG SAAAVOTAABNISSLAITKUTIVET WITHOST |
| , ,> , ,> | V . V | HHH | . 1 > 5 | F F F | I G W |
| 0 | S D I L W V. | ο Γ Γ Γ Γ Γ Γ | > > ы О о о | 3 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 | LENQTIG |
| | G S :D 1 | V R D Y A K V K D V V H I K D N I T | 0 0 0 v E v V | H H H M M M M M M M M M M M M M M M M M | 1 0 7 E |
| > | С , С Г , Г | . De par 42 | 1 F A 1. G Q V V Q P K 1 1 F A 1. G E V V S P K V V V V V V V V V V V V V V V V V V | 1 T A S Q S N L K 1 H 1 L A S H P E L T L H 1 T A K Q - Q V K L H | LANKLVSYDLEN LSNKLVYDIEN TSNKLVVYDIEN |
| = | Y V Q V Y V Q V | β.Α.Κ Θ Θ Θ | 6 6 6 1 F | 0 H 0 | A X X X |
| | Д . Ш О . Ц | F. S. S. | _ | | L V L A M A L S |
| | 20 1 20 | 300 | 9 9 9 | \times \square \times | $\Theta \cap C$ |

19 T 75

CcAPI (SEQ ID NO: 88)
AP A Thn (AY099617) (SEQ ID NO: 89)
AP A thn (BAB09356) (SEQ ID NO: 90)

Figure 21: Optimal alignment of the complete protein encoded by CcAP-1 cDNA with other homologous full-length aspartic proteinase sequences available in the NCBI.



Optimal alignment of the complete protein encoded by CcAP-2 cDNA with other homologous full-length aspartic proteinase in the NCBI